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| <b>(54) Title:</b> COMPOSITIONS AND METHODS OF TREATING IL-6 ASSOCIATED DISEASES   |  |   |
| <b>(57) Abstract</b><br><p>Transformed B cell lines secrete into a human serum culture medium under controlled conditions an inhibitor of IL-6. The cell culture medium, or purified fractions thereof having IL-6 inhibitory activity, can be administered to treat patients having disorders characterized by elevated levels of IL-6, particularly certain types of cancers, autoimmune disorders, pain associated with these disorders, side effects arising from chemotherapy, and wasting or other symptoms of certain viral diseases. The preferred cell line is RPMI 1788, from the American Type Culture Collection, although other B cell lines can also be used as a source of active material. Examples demonstrate efficacy in treatment of cancer patients and autoimmune disorders.</p> |  |   |

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## COMPOSITIONS AND METHODS OF TREATING IL-6 ASSOCIATED DISEASES

### Background of the Invention

5           The present invention relates to methods and compositions for treatment of a variety of disorders characterized by elevated IL-6 levels, and is based on materials derived from a transformed B cell line.

          Since the early 1970's, new cancer treatments have been developed based on lymphokines and modifiers of lymphokine activity. Early  
10       investigators began testing size-filtered fractions of cell supernatants and found that certain cytokines possessed the ability to influence tumor activity as well as a capability to affect the hemopoietic system, for example, as reported by McDaniel, M.C., *et al.*, *Clin. Immunol. Immunopathol.*, pp. 91 104 (1976); and Papermaster, B.W., *et al.*, *Clin. Immunol. Immunopathol.*, 5:48 - 59 (1976)). Methods used involved not  
15       only size-filtering but also lyophilization, chromatographic separation and extraction or reconstitution into various buffers. Research then focused on identifying individual components of these supernatants, as described, for example, by Papermaster, B., *et al.*, *Advances In Immunopharmacology*, pp 507 - 511 (1981); Dunn, P.A., *et al.*, *J. Immunol. Methods* 64:71 -83 (1983); Papermaster, B.W., *et al.*, *Cancer* 45:1248 -1253 (1980)).

          A number of cytokines have now been purified, cloned and expressed in recombinant hosts. Clinical trials have shown some success,  
25       however, there is toxicity in many cases at effective doses and more recent efforts have focused on searches for inhibitors of cytokines.

          It is therefore an object of the present invention to provide compositions and methods for treatment of disorders associated with elevated levels of cytokines.

30       It is a further object of the present invention to provide compositions for treatment of disorders associated with elevated levels of IL-6.

### Summary of the Invention

Transformed B cell lines secrete into a human serum culture medium under controlled conditions an inhibitor of IL-6. The cell culture medium, or purified fractions thereof having IL-6 inhibitory activity, can be administered to treat patients having disorders characterized by elevated levels of IL-6, particularly certain types of cancers, autoimmune disorders, pain associated with these disorders, side effects arising from chemotherapy, and wasting or other symptoms of certain viral diseases. The preferred cell line is RPMI 1788, from the American Type Culture Collection, although other B cell lines can also be used as a source of active material.

Examples demonstrate efficacy in treatment of cancer patients and autoimmune disorders.

### Detailed Description of the Invention

#### I. Pharmaceutical Compositions.

As used herein, "pharmaceutical compositions" include compositions containing cell secretions produced by B lymphoid cells, induced with a transforming agent such as infection with Epstein Barr virus, and cultured in a human albumin or serum based culture medium or synthetic medium, as obtained by centrifugation or filtration of the cell culture media or in purified form.

#### Cells

Cells that can be cultured to produce the pharmaceutical compositions are primarily hematopoietic cells, especially human B lymphoid cell lines transformed by Epstein Barr Virus. The most preferred cell line is RPMI 1788, deposited with the American Type Cell Culture Collection under Accession Number CCL 156, which has been previously exposed to Epstein-Barr virus. This cell line is Epstein-Barr virus nucleic antigen-positive, as is about 60% of the human population. The cell culture medium containing the secretions from these cells is

referred to herein as "LK-200", although secretions from only one cell line, ATCC CCL 156, is currently being tested clinically.

Other cell lines that can be used include EBV transformed cell lines such as IB4, Raji, which is a Burkett lymphoma cell line, and non-  
5 EBV infected B cell lines such as Bjab. These cell lines can be obtained from the ATCC or other sources, for example, Tufts New England Medical Center, Boston, Massachusetts.

#### Inducing Agents

Agents which can be used to transform the cells include viruses  
10 such as Epstein Barr virus. Inducers, which may be used alone in some cases or in combination with transforming agents, include tumor necrosis factor (TNF), endotoxin, and other agents known to those skilled in the art. In general, the cultured cells are exposed to an amount effective to  
15 activate the cells, as determined by cytokine expression, immunoglobulin secretion, and/or other indicators such as proliferation or alteration of cell surface properties or markers. In some cases, cells are initially exposed to a small amount to "prime" the cells, then to a subsequent dose to elicit greater activation of the cells. As noted above, these techniques and  
20 materials are published in the literature. The inducing agent is removed from the cell culture medium when the medium is exchanged, either through gradual supplementation or filtration or centrifugation of the cells, followed by decantation of the medium and replacement with fresh medium.

#### Cell Culture Medium

25 The cells are preferably cultured in a medium which can be administered directly to a human patient without eliciting a reaction to the medium. In the preferred embodiment, the medium is based on 2% AB human serum, for example, obtained from Bio Whittaker, Inc., Walkersville, MD, or Gibco, Life Technologies, Inc., Grand Island, NY,  
30 although albumin can be substituted for the serum immediately prior to harvesting of the cell culture medium. Human serum is placed in Iscove's Modified Dulbecco's Medium (IMDM) containing sodium

bicarbonate and 25 mM HEPES in a concentration of 2% L-glutamine. The IMDM and L-glutamine can also be obtained from Bio Whittaker.

Serum source does have an effect on cytokine levels in the final product, although it is relatively negligible. For example, the level of IL-8 in Bio Whittaker serum medium after removal of the cells (referred to as "supernatant", regardless of the method of cell removal) was measured as  $0.367 \pm 0.17$  ng IL-8/ml and the level of IL-8 in Gibco serum medium was measured as  $0.184 \pm 0.1$  ng IL-8/ml; the level of TNF in the BioWhittaker serum medium was  $0.311 \pm 0.24$  ng TNF/ml and the level of TNF in the Gibco serum medium was  $0.179 \pm 0.07$  ng TNF/ml. Cells will grow and secrete active components in albumin, but only to a limited extent.

There have been indications that the blood type of the serum source can elicit a reaction in some patients having a different blood type. Accordingly, although not a problem, it is preferred to utilize a serum source of the same blood type as the patient.

#### Culture Conditions

Cells are incubated at 37°C until they reach a minimum density of  $1 \times 10^6$  cells/ml. The pH at the time of harvest is approximately  $7.00 \pm 0.15$ .

For example, RPMI 1788 cells obtained from the ATCC are thawed in a 37°C water bath, suspended at room temperature in 0.2 micron-filtered Iscov's media containing HEPES, obtained from GIBCO BRL Life Technologies, Inc., Gaithersburg, MD, and supplemented with 2% glutamine and 2 to 5% normal human serum (Gibco) or 10 to 25% human albumin (Gibco). Cells are cultured in T75 flasks in an atmosphere of 5% CO<sub>2</sub> at 37°C until the cell density exceeds  $1 \times 10^6$  cells/ml. The cells can then be removed by centrifugation at 800 x g for 5 minutes or filtration and resuspended in T150 flasks at a density of  $0.2 \times 10^6$  cells/ml in a total volume of 200 mls of media. Cells are then cultured again until the density reaches  $1 \times 10^6$  cells/ml and the process is repeated.

Cells can then be cultured in 500 ml roller bottles, flushed with 10% CO<sub>2</sub> and sealed, at an initial cell density of  $0.2 \times 10^6$  cells/ml, until the cells reach a density of at least  $1 \times 10^6$  cells/ml.

Analysis of Cell Surface Phenotype and Cytokine Levels

- 5 Supernatants produced from RPMI 1788 (referred to herein as LK200) as well as for other cell lines: IB4, Raji, Bjab, and peripheral blood cells (PBCs) activated by exposure to a mitogen, PHA, and controls: 2% human serum medium not exposed to cells, 10% fetal calf serum (FCS) medium not exposed to cells, and a T cell line OKT3. The
- 10 results are shown in Tables 1 to 3. Table 1 demonstrates that the RPMI 1788 cells only weekly express the lambda light chain. RPMI 1788 cells labelled "ATCC" (presumably not propagated *in vitro* since the initial purchase of the line) are markedly positive for this antigen. For reasons unknown, the RPMI 1788 line does not express the "naive" CD45 isoform
- 15 found on the other lines. This line is, however, reactive with the Becton Dickinson HLe-1 mAb. According to Dr. Michel Streuli (DFCI, Boston), an expert on the alternative splice variants of CD45, the line may express an isoform containing material encoded by the B alone or the B and C exons. Regardless of the structure of this isoform, the significance of this
- 20 variant is obscure.

Table 1: SURFACE PHENOTYPE

| Marker   | <u>Cell Line</u> |     |      |      |
|----------|------------------|-----|------|------|
|          | RPMI 1788        | IB4 | Raji | Bjab |
| mAb      |                  |     |      |      |
| CD3      | -                | -   | -    | -    |
| CD5      | -                | -   | -    | -    |
| CD19     | +                | +   | +    | +    |
| CD20     | +                | +   | +    | +    |
| CD21     | 39%              | +   | +    | +    |
| HLA-DR   | +                | +   | +    | +    |
| kappa    | -                | -   | 81%  | -    |
| lambda   | 5/82             | +   | -    | -    |
| CD45     | +                | +   | +    | +    |
| (HLe-1)  |                  |     |      |      |
| CD45RA   | -                | +   | +    | 53%  |
| (Leu-18) |                  |     |      |      |
| CD45RO)  | -                | -   | -    | -    |
| (UCHL-1) |                  |     |      |      |

The (+) and (-) signs indicate 100% and 0% expression respectively.

The RPMI 1788 and IB4 cell lines are EBV+LCL. Raji is a Burkitt lymphoma and Bjab a non-EBV-infected B cell line.

All mAb used in these FACS analyses were FITC-conjugated except the anti-CD21.

The unconjugated anti-CD21 mAb was used with an anti-murine IgG-FITC second step antibody to detect CD21 surface expression.

Table 2: CYTOKINE LEVELS IN CELLS

| Supernatant                  | Cytokine Levels (pg/ml) |       |      |       |
|------------------------------|-------------------------|-------|------|-------|
|                              | IL-1 beta               | IL-2  | IL-4 | IL-6  |
| medium 2% AB serum           | 80                      | 0     | 0    | 20    |
| medium 10% FCS               | 80                      | 0     | 0    | 92    |
| PHA-activated PBMC           | 1,003                   | 1,602 | 13   | 4,705 |
| IB4                          | 80                      | 0     | 0    | 20    |
| Raji                         | 80                      | 0     | 0    | 20    |
| Bjab                         | 80                      | 0     | 0    | 20    |
| RPMI 1788                    | 80                      | 0     | 0    | 94    |
| (from ATCC)                  |                         |       |      |       |
| RPMI 1788 (from 2/16 freeze) | 80                      | 0     | 0    | 94    |
| LK200 (from Woburn) 4/23     | 80                      | 0     | 0    | 20    |
| LK200 (from Woburn, 1/8)     | 80                      | ND    | ND   | 20    |
| LK200 (from Woburn, 1/10)    | 80                      | ND    | ND   | 20    |

\* *IL-1 beta, TNF, IL-6, IL-10, IL-1RA, soluble IL-1 receptor type II (sIL-1RII), and IL-8 were assayed by RIA. IL-2, IL-4, GM-CSF, and IFN-gamma were assayed by ELISA with kits obtained from Endogen. IL-12 was similarly assayed with a kit from R&D Systems.*

Table 2 continued:

| Supernatant                  | Cytokine Levels (pg/ml) |       |       |           |
|------------------------------|-------------------------|-------|-------|-----------|
|                              | IL-8                    | IL-10 | IL-12 | IFN-gamma |
| medium 2% AB serum           | 34                      | 20    | 0     | 0         |
| medium 10% FCS               | 41                      | 29    | 0     | 0         |
| PHA-activated PBMC           | 22,640                  | 1,436 | 0     | 865       |
| IB4                          | 30                      | 20    | 0     | 0         |
| Raji                         | 30                      | 22    | 0     | 0         |
| Bjab                         | 36                      | 920   | 0     | 0         |
| RPMI 1788(from ATCC)         | 135                     | 20    | 0     | 0         |
| RPMI 1788 (from 2/16 freeze) | 34                      | 20    | 0     | 0         |
| LK200 (from Woburn) 4/23     | 30                      | 20    | 0     | 0         |
| LK200 (from Woburn, 1/8)     | 96                      | 20    | ND    | ND        |
| LK200 (from Woburn, 1/10)    | 83                      | 27    | ND    | ND        |

\* > highest concentration used in the generation of the standard curve.

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Table 2 continued:

| Supernatant                  | Cytokine Levels (pg/ml) |       |        |          |
|------------------------------|-------------------------|-------|--------|----------|
|                              | IL-1RA                  | TNR   | GM-CSF | sIL-1RII |
| medium 2% AB serum           | 107                     | 41    | 0      | 432      |
| medium 10% FCS               | 93                      | 83    | 0      | 164      |
| PHA-activated PBMC           | 8,516                   | 2,181 | 190    | 528      |
| IB4                          | 48                      | 178   | 0      | 1,100    |
| Raji                         | 86                      | 98    | 0      | 212      |
| Bjab                         | 227                     | 113   | 0      | 148      |
| RPMI 1788 (from ATCC)        | 86                      | 191   | 0      | 404      |
| RPMI 1788 (from 2/16 freeze) | 141                     | 118   | 0      | 296      |
| LK200 (from Woburn) 4/23     | 61                      | 175   | 0      | 304      |
| LK200 (from Woburn, 1/8)     | 137                     | 225   | ND     | 236      |
| LK200 (from Woburn, 1/10)    | 49                      | 252   | ND     | 352      |

The results shown in Table 2 demonstrate that LK200 and RPMI 1788 supernatants contain modest amounts of IL-8 (consistently less than 135 pg/ml) and modest amounts of TNF (less than 250 pg/ml). Supernatant from the Bjab line contains a great deal of IL-10 (920 pg/ml). This is the one B cell line studied that is not EBV-infected. The IB4 line sheds the type II IL-1 receptor (1,100 pg/ml). The Bjab line secretes a modest amount (227 pg/ml) of the IL-1RA.

Table 3: CYTOKINE INDUCTION IN PBMC\*

| Inducer  | Cytokine levels (pg/ml) |       |      |       |
|--|-------------------------|-------|------|-------|
|  | IL-1 beta               | IL-2  | IL-4 | IL-6  |
| medium 2% AB serum                                 | 143                     | 0     | 0    | 3,475 |
| PHA  | 1,003                   | 1,602 | 13   | 4,705 |
| LK200 (from Woburn) 1:3 <sup>+</sup>               | 80                      | 0     | 0    | 2,368 |
| LK200 1:6 <sup>+</sup>                             | 80                      | 0     | 0    | 1,147 |
| RPMI 1788 (from ATCC) supernatant 1:3 <sup>+</sup> | 80                      | 0     | 0    | 925   |
| RPMI 1788 (from ATCC) supernatant 1:6 <sup>+</sup> | 80                      | 0     | 0    | 483   |
| OKT3 (0.1 µg/ml)                                   | 102                     | 0     | 0    | 2,852 |
| OKT3 + LK200 (from Woburn) 1:3 <sup>+</sup>        | 80                      | 0     | 0    | 2,722 |
| OKT3 + LK200 1:6 <sup>+</sup>                      | 1,032                   | 0     | 0    | 4,004 |

\* The donor of the PBMC for this experiment (5/9/95) was Damien Sorce. For each condition tested,  $12.5 \times 10^6$  cells were incubated in 5 ml total volume in 50 ml polypropylene centrifuge tubes which had been previously coated with human albumin (10 µg/ml x 1 hr). Cells were cultured in RPMI medium supplemented with 2% human AB serum and 4 µg/ml polymyxin B. After 48 hrs at 37°C, the PBMC were vortexed briefly to ensure equilibration of the secreted cytokines throughout the culture medium and then pelleted by centrifugation. The supernatants were removed and frozen under sterile conditions for future analysis. IL-1 beta, TNF, IL-6, IL-10, IL-1RA, soluble IL-1 receptor type II (sIL-1RII), and IL-8 were assayed by RIA. IL-2, IL-4, GM-CSF, and IFN-gamma were assayed by ELISA with kits obtained from Endogen. IL-12 was similarly assayed with a kit from R&D Systems.

\* refers to final dilution in culture medium.

Table 3 continued:

| Inducer                                | Cytokine levels (pg/ml) |       |       |           |
|--|-------------------------|-------|-------|-----------|
|  | IL-8                    | IL-10 | IL-12 | IFN-gamma |
| medium 2% AB serum                     | 22,830                  | 45    | 0     | 5         |
| PHA                                    | 22,640                  | 1,436 | 0     | 865**     |
| LK200 (from Woburn) 1:3*               | 25,140                  | 38    | 0     | 6         |
| LK200 1:6+                             | 25,980                  | 32    | 0     | 0         |
| RPMI 1788 (from ATCC) supernatant 1:3+ | 16,640                  | 49    | 0     | 0         |
| RPMI 1788 (from ATCC) supernatant 1:6+ | 13,270                  | 37    | 0     | 0         |
| OKT3 (0.1 µg/ml)                       | 18,180                  | 543   | 0     | 461       |
| OKT3 + LK200 (from Woburn) 1:3+        | 16,320                  | 561   | 0     | 256       |
| OKT3 + LK200 1:6+                      | 28,970                  | 1,013 | 0     | 587**     |

\* *refers to final dilution in culture medium*

\*\* *>highest concentration used in the generation of the standard curve*

Table 3 continued:

| Inducer                                | Cytokine levels (pg/ml) |       |        |          |
|--|-------------------------|-------|--------|----------|
|  | IL-1RA                  | TNF   | GM-CSF | sIL-1RII |
| medium 2% AB serum                     | 4,165                   | 292   | 0      | 488      |
| PHA                                    | 8,516                   | 2,181 | 190    | 528      |
| LK200 (from Woburn) 1:3+               | 2,372                   | 223   | 0      | 316      |
| LK200 1:6+                             | 2,906                   | 159   | 0      | 356      |
| RPMI 1788 (from ATCC) supernatant 1:3+ | 6,277                   | 198   | 0      | 365      |
| RPMI 1788 (from ATCC) supernatant 1:6+ | 4,811                   | 154   | 0      | 344      |
| OKT3 (0.1 µg/ml)                       | 6,328                   | 541   | 2      | 524      |
| OKT3 + LK200 (from Woburn) 1:3+        | 7,755                   | 651   | 2      | 668      |
| OKT3 + LK200 1:6+                      | 4,545                   | 1,375 | 19     | 524      |

5 As demonstrated by Table 3, although the various LK200  
preparations alone did not induce PBMC to generate appreciable amounts  
of any cytokine, they amplified the inductive effects of low concentrations  
of the anti-TCR mAB OKT3. Specifically, these preparations enhanced  
IL-1 beta production by as much as 10-fold over that induced by OKT3  
10 alone. TNF production was augmented 250% and that of IL-8 by 50%.  
IL-10 production was doubled. GM-CSF production was increased from  
2 pg/ml with OKT3 alone to 19 pg/ml with OKT3 + LK200 (1:6  
dilution).

The 2% human AB serum used in the generation of LK200 itself  
15 induces an abundance of IL-6, IL-8, and IL-1RA, presumably due to FcR  
signalling. LK200 and RPMI 1788 supernatants markedly suppress this  
effect. IL-6 production in particular was reduced by greater than 80%.  
IL-8 production was reduced by approximately 40%.

These results demonstrate that the composition not only serves as  
20 an inhibitor but is more effective when diluted to lower concentrations,

presumably as a result of inhibition or competitive interactions with other components of the composition.

#### Pharmaceutically Acceptable Carriers

5 The pharmaceutical composition can consist of the culture medium alone or diluted with deionized water or normal saline (0.15 N NaCl) or other physiological buffer. Care should be taken to avoid changes in pH. The medium should not be diluted until immediately prior to administration to the patient.

#### Processing of Cell Culture Medium

10 In the preferred embodiment, the cells are removed from the culture medium to yield a supernatant containing the biologically effective molecules. Cells can be removed by centrifugation or filtration. Typically, the medium is withdrawn from the roller bottles by decantation, pipetting, filtration or centrifugation, then the medium is placed into sterile syringes. Further processing of the material can be obtained based on separation by molecular weight, chromatography on ion-exchange resins, and other methods known to those skilled in the art, where the active fraction is defined by inhibition of IL-6 activity.

#### Sterilization

20 Sterility can be assured by culturing the cells under standard sterile conditions, with removal of the cell culture medium using aseptic conditions. Alternatively, the cell culture medium can be purified by filtering with a filter not excluding molecules of less than 250,000, or more preferably, not less 150,000 daltons, for example, a 0.25 micron filter. The culture medium may also be sterilizable using gamma irradiation.

#### Storage

30 The supernatant, once removed from 37°C temperature and 10% nitrogen atmosphere of the cell culture bottles and separated from the cells, can be maintained at room temperature for at least two to four hours without any loss of activity. For longer periods of storage, the medium should be rapidly frozen (defined as in less than two hours) in

liquid nitrogen, on dry ice, or in an ultrafreezer, at a temperature of about -40°C to -80°C.

Analysis of Pharmaceutical Composition

Assays on 45 lots of LK-200 for various cytokines indicate that

5 concentrations are in the nanogram per milliliter range, as shown in Table 2. All lots showed essentially no batch to batch variation. There is some direct correlation with levels of cytokines with human serum

concentration in the media. The endotoxin levels in the supernatants have consistently measured at or less than 10 pg endotoxin/ml. In general,

10 levels up to 10 ng are acceptable for administration to a patient.

## II. Methods of Treatment.

### Disorders which can be Treated

Interleukin-6 (IL-6) is a pluripotent cytokine that has an effect on a wide array of different cell types. It is generally recognized as a B-cell growth factor, originally being identified as an inducer of immunoglobulin secretion in the Epstein-Barr Virus (EBV) transformed CESS lymphoblastoid line. Although it is classified as an acute phase protein, its role is multifaceted and remains somewhat cloudy. Clearly, it is an important cytokine thought to be intimately involved in several pathologies.

The receptor for IL-6 is gp130. This is a transmembrane, transducing receptor which spans the cell membrane and signals the cell once the ligand is bound. Sharing the gp130 receptor with IL-6 are other cytokines including Leucocyte Inhibitory Factor (LIF), Oncostatin M (OSM), IL-11 and Ciliary Neurotropic Factor (CNTF).

Inhibitors of IL-6 are retinoic acid and some steroids (dexamethasone). Soluble gp130 at one time had been considered to be inhibitory for IL-6 activity. Binding of IL-6 with soluble gp130, however, exacerbates its pathological effects, particularly osteoclast formation. In some diseases, multiple myeloma and Kaposi's sarcoma for example, IL-6 is thought to play an autocrine or paracrine role. In these cases, IL-6 is both generated and then used by the same or adjacent cells, perpetuating the disease.

Pathologies where IL-6 has been implicated include the following:

Multiple myeloma (mm); Prostate cancer; Ovarian cancer; Castleman's disease; Kaposi's sarcoma (KS); Monoclonal gammopathy; Mesangial proliferative glomerulonephritis; Rheumatoid arthritis and other arthritides (RA); Systemic Lupus Erythematosus (SLE); Multiple sclerosis (MS); Myasthenia gravis (MG); Guillain-Barre syndrome (GBS); Toxic shock syndrome; Bacterial and viral meningitis; HTLV-1-associated myelopathy (HAM); Alzheimer's disease; Osteoporosis (osteoclastic disease); Cardiac myxoma and Hypercalcemia. Additional pathologies

where IL-6 may be implicated included Allergies; Common variable immunodeficiency; Anorexia nervosa; Inflammatory bowel disease; Critical illness polyneuromyopathy; Acute pyelonephritis; Autoimmune hepatitis; Large-cell lymphoma; Alveolar echinococcosis; Endometriosis; 5 Atherosclerosis; Psoriasis; Human Parvovirus infection; Renal cell carcinoma; Preeclampsia; Cholangiocarcinoma; HIV; Acoustic neuromas; Hairy-cell leukemia; Necrotizing enterocolitis; Ulcerative colitis; Bronchopulmonary dysplasia; Schizophrenia and affective disorders; Retinoblastoma; Pituitary tumors; Acute pyelonephritis; Thyroid 10 carcinoma; Paraganglioma; Candida albicans infection; Giant cell arteritis; Psoriasis; Melanoma; Chronic pancreatitis; Carpal tunnel syndrome; Interstitial cystitis; Astrocytoma; Respiratory syncytial virus infection; Liver transplantation; Meningiomas; Gastric carcinoma; Sarcoidosis; Uterine cancer; Pulmonary Large cell carcinoma; *Helicobacter pylori* 15 infections; Ductal Breast carcinoma; Granulomas of giant cell arteritis; Colorectal cancer; Clinical chorioamnionitis; non-Hodgkin's lymphoma; Thyrotoxicosis; Osteosarcoma; Malignant mesothelioma; Typhoid fever; IgA nephropathy; Acute pancreatitis; Fanconi's anemia; Paraneoplastic thrombocytosis; and Cystic fibrosis.

#### 20 Dosages and Administration Schedules

Dosages and administration schedules are highly individual and are optimized individually in response to alleviation of clinical symptoms, for example, reduction in tumor mass, relief from bone pain, and other subjective or objective criteria.

#### 25 Cancers and Viral Diseases

In the treatment of cancers or viral infections, a number of different criteria can be used as indicia of efficacy. For example, tumor size can be monitored by standard tumor detection and measurement methods such as CAT scanning (computerized axial tomography), MRI 30 (magnetic resonance imaging), and nuclear medicine scans, as known to those of skill in the art. Progression and involvement of lesions caused by a virus or total anatomic involvement of the disease, which are herein

used as indicators of the progression of the disease, can be determined by standard methods known in the art for each specific lesion type, depending upon the specific disease, for example, organ function or antibody titers. For treatment of viral infections, effectiveness can be measured as a reduction in the intensity and duration of the symptoms of the invention. This can include measurement of pathologic and pathophysiologic activities that are reduced, for example, elevated liver functions, elevated bilirubin levels, enlarged liver size and enlarged spleen.

#### *Reduction in Pain and Side Effects of Chemotherapy*

The composition is also effective in reducing side effects of chemotherapy and radiation therapy. Side effects which can be reduced include nausea, vomiting and hair loss. Pain is also reduced in many cases. Pain remission can include remission of pain from a decrease in tumor size or in space-occupying lesions, thus decreasing organ pressure and compression of anatomical structures (i.e., nerves, vessels and other organs), as well as remission in pain not associated with a decrease in tumor size or a decrease in lesions, such as pain in bones and other pain remission that occurs before a significant decrease in tumor size or lesions occurs.

#### *Treatment of Autoimmune Disorders*

Autoimmunity is described as an immune response mounted against self-components which ultimately results in pathogenic consequences. Diseases which result from autoimmune responses are widespread and varied in clinical presentation. One common factor shared by many of these disease entities is the lack of a known etiologic agent or triggering event for the production of these aberrant responses. Rheumatological illnesses encompass a large number and wide spectrum of different autoimmune diseases, such as rheumatoid arthritis, scleroderma, dermatomyositis, polymyositis, discoid lupus erythematosus, Sjogren's syndrome and systemic lupus erythematosus. For the most part, the etiologies and pathogenic mechanisms of these disorders are still

unknown. One common theme in many of these maladies is the presence of substantial quantities of antibodies immunoreactive with self-components. One example of such a rheumatic disease is systemic lupus erythematosus. One characteristic shared by many of these patients is the presence of high titers of autoantibodies in their sera. These autoantibodies may be directed against a myriad of host components, such as ribonucleoproteins (Sm, nRNP, Ro and La), DNA, RNA, histones, erythrocytes, and immunoglobulin, as well as other characterized and uncharacterized autoantigens. Myasthenia Gravis is a disease of unknown etiology, characterized by circulating antibodies to acetylcholine receptors. The disease is manifested by muscle weakness with a predilection for ocular and other cranial muscles. It has a tendency to fluctuate in severity. There are no signs of neurological lesions. The disease is believed to have an immunological basis and anticholinergic antibodies binding to acetylcholine receptors are found in most patients with the disease. Patients may also present with anti-muscle antibodies. The anticholinergic antibodies effectively reduce the number of functional acetylcholine receptors. It is also believed that cellular immune activity against receptors has been found. Patients exhibit generalized weakness that may be fluctuating, most commonly in the use of voluntary muscles. Symptoms such as diplopia, ptosis, and dysphasia are noted. Activity increases weakness of affected muscles. In some cases, efficacy will be demonstrated by reduction in autoantibody levels, in other cases by a decrease in the severity of symptoms. For example, efficacy can be demonstrated by an improvement in large muscle and proximal muscle dysfunction, a reduction in anti-acetylcholine activity, reduction in anti-smooth muscle antibody levels, and improvement in swallowing dysfunctions and dysphasia.

#### Dosages

The general method of administration and dose schedule for LK-200 is as follows. Frozen LK-200 is thawed in less than two hours, without excessive heating or mechanical agitation. 50 cc of the LK-200 is

then mixed with 50 to 100 cc of normal saline. In some instances, depending upon disease or response, 100 to 200 cc of LK-200 may be mixed with 50 to 100 cc of sterile normal saline. In general, 50 cc mixed with 50 to 100 cc of Sterile Normal Saline is administered once daily for the first 10 to 14 days and then on a three times a week schedule. In some circumstances, up to 200 cc of LK-200 mixed with 50 to 100 cc sterile normal saline is administered four times a day. The mixture is infused intravenously over a 5 to 45 minute period, depending upon the total volume to be administered. A slower rate is sometimes used if there is a possibility of cardiac decompensation in a patient.

As demonstrated by the data in Table 3, and by preliminary clinical data in which efficacy has been enhanced by lowering the dosage from 50 mls cell culture supernatant administered three to five times per week to ten or 25 mls, there is a negative dose dependency of the inhibitor of IL-6 in the composition.

The intravenous administration can be accomplished peripherally through normal intravenous injection. In some cases patients have specialized ports, such as a Hieman Catheter, which can be used for infusion. This includes certain sub-clavian catheters which are used for chemotherapy and or hyperalimentation or other accepted uses of these catheters. The LK-200 can also be administered via a direct intra-arterial infusion on a selective basis, or via intra-peritoneal infusion. Portable infusion pumps may be preferred in some cases.

Dosages can be varied from one to four doses daily or decreased to one every other day or two or three times a week. Dosages typically will be equivalent to between 0.1 and 100 cc/day, averaging about 10 to 50 cc administered once or twice a day or every other day.

Of course, dosages as provided above are based on administration of the cell culture medium. In the event that the purified fractions are used, the dosage will be adjusted accordingly.

The present invention will be further understood by reference to the following non-limiting examples.

**Example 1: Treatment of Cancer Patients.**

Day 1 through Day 7: One daily dose of 10 - 50 ml LK 200 was administered intravenously in 100 ml normal saline, over 15 to 30 minutes.

5 Day 8 forward: 10 - 50 ml LK 200 was administered two to three times per week.

Increases in any given dosage, if tumor response was not satisfactory, were found to be better accomplished by increasing the number of doses per day, rather than the amount in a single dose. No  
10 adverse reactions were observed from additional doses.

**Patient condition**

Upon administration of the first dose of LK 200, patients experienced the following immediate effects, i.e., within about 24 - 48 hours, in addition to the immediate tumor shrinkage detailed in Table 4:  
15 remission of pain; increase in appetite; increase in energy, halting of wasting; increase in quality of sleep.

Table 4 provides data from patients treated with LK 200, detailing the regression of specific tumors or lesions associated with the listed disease. Tumors and lesions were measured by CAT scan, MRI  
20 and visual inspection.

**TABLE 4: Effect of Treatment of Cancer Patients**

| <b>PATIENT</b> | <b>TYPE OF CANCER</b>   | <b>LENGTH OF</b> | <b>% REGRESSION</b>    |
|----------------|---|------------------|------------------------|
| A101           | Non-Hodgkins Lymphoma   | 8 weeks          | 68%                    |
| A102           | Non-Hodgkins Lymphoma   | 4 weeks          | 30%                    |
| A103           | Mesothelioma, metastatic  | 1 week           | 12%                    |
| A104           | Squamous Cell Carcinoma of the Brain                              | 3 weeks          | 28%                    |
| A105           | Embryonic Carcinoma of the Testicle                               | 3 weeks          | 36%                    |
| A106           | Carcinoma of the Right Breast                                     | 6 weeks          | 35%                    |
| A107           | Carcinoma of the Breast-S/P<br>Mastectomy; Bone Metastasis        | (new)            |                        |
| A108           | Carcinoid, metastatic   | 8 weeks          | 35% tumor now cystic   |
| A109           | Malignant Melanoma Cerebellum<br>of the Brain: Cerebrum           | 8 weeks          | 78% tumor necrotic     |
|                |   | 8 weeks          | 52% tumor necrotic     |
| A110           | Ovarian Carcinoma   | 3 weeks          | 35% estimated          |
| A111           | Ovarian Carcinoma   | 2 weeks          | 40% tumor now operable |
| A112           | Malignant Melanoma-metastatic (liver)<br>to Bone and Liver (bone) | 2 weeks          | 50%                    |
|                |   |                  | bone pain free         |
| V101           | Chronic Active Hepatitis  | 8 weeks          | 40%                    |
| V102           | CA of Lung  | 2 weeks          | pending                |
| V103           | Psoriasis   | 1 week           | 10% estimated          |
| V104           | CA of Larynx-S/P Laryngectomy;<br>Sub-cranial metastatic disease  | 4 weeks          | 25% (with lysis)       |
| V105           | Kaposi's Sarcoma  | 8 weeks          | 100%                   |
| R101           | Kaposi's Sarcoma (HIV<br>Immunocompromised)                       | 8 weeks          | 60%                    |

**\*Additional patient data:****Patient A112 (malignant melanoma metastatic to bone and liver)**

|                                     |  |
|-------------------------------------|--|
| Prior treatment:                    | Extensive disease of bone and liver<br>Severe bone pain  |
| Immediate effect of treatment:      | pain free  |
| After one week of standard therapy: | Liver functions, alkaline phosphatase and BUN rose dramatically; creatinine remained essentially normal; sternal bone lesion resolved 60% (estimated); Liver cleared approximately 50% |
| Resolution:                         | Family decided to stop treatment. Patient from was believed to be Tumor Necrosis Syndrome. Due to family's decision, dialysis was not tried.   |

**Patient V101 (chronic active hepatitis):**

|                                      |   |
|--------------------------------------|---|
| Began treatment April 1993           |   |
| Prior to treatment :<br>(April 1993) | Bilirubin 6.7<br>Liver functions: elevated<br>Patient icteric   |
| On June 1, 1993:                     | Bilirubin 4.5<br>Liver functions: normal<br>Patient anicteric<br>Liver: 10% reduction in size<br>Spleen: 25-30% reduction in size |

**Patient V103 (psoriasis):**

|                               |   |
|-------------------------------|---|
| Initial response to treatment | Moderate decrease in discomfort; reddened and indurated patches decreased; palmer lesions disappeared; patient could stop cortisone treatment for several days and now used 25% of the original dose. |
|-------------------------------|---|

**Patient V105 (Kaposi's Sarcoma):**

After one month of therapy, the largest lesion disappeared and the remaining four satellite lesions regressed 30%. One month after treatment was stopped, all lesions had disappeared.

**Patient R101 (Kaposi's Sarcoma):**

|                                    |  |
|------------------------------------|--|
| Prior record:                      | immunocompromised male due to HIV; CD4 counts less than 25; prior treatment included interferon 3 times per week for 1 year, with no regression of the multiple lesions. |
| After 8 weeks of standard therapy: | larger lesions have reduced by 40-50%; several of the flat skin lesions have regressed 60%   |

**Example 2: Treatment of patients with psoriasis.**

5 Psoriatic keratinocytes (skin cells) produce and respond to IL-6.  
(Grossman, R., et al. "Interleukin-6 is expressed in high levels in  
Psoriatic Skin and Stimulates Proliferation of Cultured Keratinocytes".  
10 Proc. Natl. Acad. Sci. USA 1986: 6367, 1989; Bergui, L., "Interleukin-  
3 and Interleukin-6 Synergistically Promote the Proliferation of Malignant  
Plasma Cell Precursors in Multiple Myeloma". J. Exper. Med. 170:613,  
1989.) As shown below, LK-200 administered to patients with psoriasis  
showed resolution of the condition. The disruption of IL-6 production  
and/or action could account for these observations.

15 A patient with severe psoriasis who was taking 20 grams of  
hydrocortisone topically a day was started on LK-200, as described  
above. Since hydrocortisone has numerous undesirable side effects it was  
highly desirable to decrease this dosage, as well as to increase efficacy of  
the treatment.

20 After four weeks the patient was using only 5 grams of  
hydrocortisone a day with about 30% resolution of her psoriasis.

A second patient with psoriasis associated with malignant myeloma  
was treated with LK-200. The psoriasis had been unresponsive to  
traditional medical treatment.

25 Over a four week period the patient's IgG level from 21 gm% to  
15 gm%. The psoriasis resolved 50% in the first two or three weeks.  
The patient had a significant reduction in bone pain. The patient stopped  
using narcotics and other strong analgesia.

**Example 3: Treatment of patient with Rheumatoid Arthritis.**

30 LK-200 suppresses the generation of inflammatory cytokines. The  
erythrocyte sedimentation rate (ESR) of patients with the AIDS wasting  
syndrome which were serially obtained and fell dramatically after  
initiating LK-200 treatment. The sedimentation rate in other patients  
being treated with LK-200 have also fallen dramatically regardless of the  
type of tumor or underlying immunologic disease. The sed rate (ESR) is  
35 a crude index of the severity of an ongoing inflammatory disorder. It is

largely determined by the plasma level of fibrinogen, a coagulation protein produced in the liver. The production of fibrinogen is regulated by IL-6. A precipitous decline in the ESR could therefore be due to the suppression of IL-6 production or to interference with its biologic effects.

5        In view of these results, a middle aged female with severe Rheumatoid Arthritis who was refractory to all forms of conventional therapy including gold treatments and methotrexate therapy was treated with LK-200. The patient had a sed rate of 60 at the start of therapy. LK-200 was administered for two weeks.

10        After two weeks of therapy, the patient's sed rate had returned almost to normal at around 15-20. She had no further pain in her hands and she was able to resume household chores.

**Example 4:        Treatment of female patient with myasthenia gravis.**

**Patient:** The patient is a thirteen year old white female with an  
15        established diagnosis of Myasthenia Gravis. She was in an extremely weakened condition, in a wheel chair, unable to lift her head, and unable to easily move any of her large peripheral muscle groups. She had a strongly positive Hess test for ocular paralysis. She had extreme ptosis, with moderate diplopia. The patient had a peak flow of 325 cc prior to  
20        the start of therapy. She was maintained on Mestinon 60 milligrams every two hours during the day and 180 milligrams at bedtime.

**Method:** Sterile thawed LK-200 was administered i.v. to the patient over a period of 20 to 45 minutes. 50 cc of LK-200 was mixed with in 50 cc of sterile normal saline and prepared for infusion by peripheral  
25        intravenous injection. The IV solution was administered to the patient daily on Monday through Friday for two weeks.

During the first two week period the patient experienced some mild improvement in peripheral muscle strength and some slight reduction ptosis of the eye. The diplopia was somewhat better. Additionally it was  
30        noted that her Mestinon dosage during waking hours could be reduced from 60 milligrams every two hours to an occasional thirty milligrams every two hours. This was sporadic.

Since pulse therapy is sometimes effective with medications used in treatment of Myasthenia Gravis it was decided to change her regimen to 100 cc LK-200 in 50 cc of normal saline infused over 30 minutes to be given to her every other day. Within seven days the patient had a

5 significant response. She no longer took afternoon naps, her muscle strength increased, her diplopia resolved entirely, and she was able to rise from the wheel chair and walk unassisted. The Mestinon dosage could be reduced to 30 milligrams every two hours and occasionally omitted. The

10 peak flow initially went from 325 cc down to 220 cc. Over the next three to four week period, peak flow consistently rose and is now over 350-400 cc. The patient now walks unassisted, her Hess test is entirely negative, and initial observations of muscle weakness have been resolved. She continued to improve steadily at the sixth week of treatment. Dosing of LK-200 was then reduced to 100 cc Monday, Wednesday and Friday.

15 Measurements of acetylcholine antibodies and anti-smooth muscle antibodies further demonstrate efficacy. After ten days of treatment with LK-200, the level of acetylcholine receptor antibodies was 3.9 nmols/L. After an additional month of treatment the level had decreased to 3.0 nmols/L.

I claim:

1. A method of treating a patient having a disorder characterized by elevated IL-6 levels comprising administering to the patient an effective dosage of a composition to decrease IL-6 levels, wherein the composition consists of a sterile culture medium in which transformed B cells have secreted immunomodulators or a purified fraction thereof having IL-6 inhibitory activity.
2. The method of claim 1 wherein the dosage is equivalent to less than 50 mls of culture medium administered twice daily.
3. The method of claim 1 wherein the disorder is a cancer other than solid tumors characterized by elevated levels of IL-6.
4. The method of claim 1 wherein the disorder is an autoimmune disorder.
5. The method of claim 1 wherein the disorder is symptoms arising from treatment with chemotherapeutic agents.
6. The method of claim 1 wherein the disorder is pain.
7. The method of claim 1 wherein the disorder is selected from the group consisting of Multiple myeloma (mm); Prostate cancer; Ovarian cancer; Castleman's disease; Kaposi's sarcoma (KS); Monoclonal gammopathy; Mesangial proliferative glomerulonephritis; Rheumatoid arthritis and other arthritides (RA); Systemic Lupus Erythematosus (SLE); Multiple sclerosis (MS); Myasthenia gravis (MG); Guillain-Barre syndrome (GBS); Toxic shock syndrome; Bacterial and viral meningitis; HTLV-1-associated myelopathy (HAM); Alzheimer's disease; Osteoporosis (osteoclastic disease); Cardiac myxoma and Hypercalcemia. Additional pathologies where IL-6 may be implicated included Allergies; Common variable immunodeficiency; Anorexia nervosa; Inflammatory bowel disease; Critical illness polyneuromyopathy; Acute pyelonephritis; Autoimmune hepatitis; Large-cell lymphoma; Alveolar echinococcosis; Endometriosis; Atherosclerosis; Psoriasis; Human Parvovirus infection; Renal cell carcinoma; Preeclampsia; Cholangiocarcinoma; HIV; Acoustic neuromas; Hairy-cell leukemia; Necrotizing enterocolitis; Ulcerative

colitis; Bronchopulmonary dysplasia; Schizophrenia and affective disorders; Retinoblastoma; Pituitary tumors; Acute pyelonephritis; Thyroid carcinoma; Paraganglioma; Candida albicans infection; Giant cell arteritis; Psoriasis; Melanoma; Chronic pancreatitis; Carpal tunnel syndrome; Interstitial cystitis; Astrocytoma; Respiratory syncytial virus infection; Liver transplantation; Meningiomas; Gastric carcinoma; Sarcoidosis; Uterine cancer; Pulmonary Large cell carcinoma; *Helicobacter pylori* infections; Ductal Breast carcinoma; Granulomas of giant cell arteritis; Colorectal cancer; Clinical chorioamnionitis; non-Hodgkin's lymphoma; Thyrotoxicosis; Osteosarcoma; Malignant mesothelioma; Typhoid fever; IgA nephropathy; Acute pancreatitis; Fanconi's anemia; Paraneoplastic thrombocytosis; and Cystic fibrosis.

8. The method of claim 1 wherein the cells are transformed by infection with Epstein-Barr virus or a portion thereof.

9. The method of claim 1 wherein the cell line is RPMI 1788 deposited with the American Type Cell Culture under Accession Number CCL 156.

10. A composition for treating a disorder characterized by elevated IL-6 comprising an effective amount of a composition to decrease IL-6 levels in a patient in need of treatment thereof, wherein the composition consists of a sterile cell culture medium in which transformed B cells have secreted immunomodulators or a partially purified fraction thereof having IL-6 inhibitory activity in a pharmaceutically acceptable carrier.

11. The composition of claim 10 wherein the dosage is equivalent to less than 50 mls of culture medium administered twice daily.

12. The composition of claim 10 wherein the disorder is a cancer characterized by elevated levels of IL-6.

13. The composition of claim 10 wherein the disorder is an autoimmune disorder.

14. The composition of claim 10 wherein the disorder is symptoms arising from treatment with chemotherapeutic agents.

15. The composition of claim 10 wherein the disorder is pain.

16. The composition of claim 10 wherein the cells are transformed by infection with Epstein-Barr virus or a portion thereof.

17. The composition of claim 10 wherein the cells are of cell line RPMI 1788 deposited with the American Type Cell Culture under Accession Number CCL 156.

18. The composition of claim 10 wherein the cell culture medium is human serum.

19. The composition of claim 10 wherein the cell culture medium is human albumin.

20. The composition of claim 10 wherein the composition is purified based on molecule weight and ion-exchange chromatography.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/08679

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C12P1/00 A61K38/17 //(C12P1/00,C12R1:91)

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12P C12R C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| X          | EP,A,0 448 181 (GIST-BROCADES N.V.) 25<br>September 1991<br>see column 2, line 16 - line 27; claims;<br>example 1<br>see column 4, line 47 - line 56<br>see column 8, line 33 - line 41<br>--- | 1-20                  |
| P,X        | EP,A,0 696 594 (BAYER CORPORATION) 14<br>February 1996<br>see page 7, line 6 - line 39; claims<br>-----  | 1-20                  |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

23 September 1996

Date of mailing of the international search report

02 10. 96

Name and mailing address of the ISA

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/08679

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 1 - 9 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter: nal Application No

PCT/US 96/08679

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| EP-A-448181                               | 25-09-91            | JP-A- 8099996              | 16-04-96            |
| EP-A-696594                               | 14-02-96            | US-A- 5527546              | 18-06-96            |
|   |                     | AU-A- 2838995              | 22-02-96            |
|   |                     | CA-A- 2155629              | 11-02-96            |
|   |                     | JP-A- 8169840              | 02-07-96            |